TS043
Modulation of adipose tissue derived stem cells encapsulated in injectable multi-functional hydrogels

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Objective: The cellular microenvironment plays an important role in controlling the cellular behavior. Therefore, in Tissue Engineering strategies, systems have been designed so that by combining cells, signaling molecules and engineered substrates, one can mimic as closely as possible the native microenvironment of the extracellular matrix. In this context, injectable hydrogels have been developed that allow controlling the cellular spatial distribution and that provide a 3D support. However, many of these systems fail to replicate the mechanical properties of tissues and more significantly to provide a cell-friendly environment. Therefore, the aim of this study is to evaluate the cellular response of human adipose stem cells (hACS) within a growth factor-enriched injectable hydrogel.
Methods: Hydrogels were formed by combining platelet lysates (PLs) with a methacrylated gellan gum (MeGG) solution at different ratios. The hydrogels were further stabilized by photopolymerization. The parameters of photocrosslinking were varied and the dynamical mechanical properties evaluated for all the conditions. Total protein content released from the hydrogels was quantified for all the conditions using micro-BCA assay. Human ASCs were both seeded on the surface of the hydrogels for 7 days and encapsulated within the materials for 14 days.
Results and Discussion: Hydrogels with tunable mechanical properties were fabricated by changing the volume ratio of PLs and MeGG. The highest elastic modulus (nearly 500 kPa) was achieved for the condition with the lowest volume of PLs (2MeGG:1PLs), being significantly more elastic than MeGG alone. The rate of release of proteins present in the PLs from the PLs-MeGG hydrogels was higher for the condition with equal volume of MeGG and PLs (1MeGG:1PLs), as a result of the lower crosslinked polymer network. Human ASCs cultured onto the engineered surfaces showed a good metabolic activity and proliferation. Immunostaining revealed that MeGG:PL combinations fostered the attachment and spreading of cells. A strikingly improved cellular behavior was observed for the formulations with PLs, when compared to MeGG alone. This behavior was further confirmed at a 3D scale, demonstrated by a significantly higher cellular metabolic activity on the hydrogels with PLs.
Conclusion: Our system combines the mechanical support from MeGG with the biological cues from PLs to engineer a cell-friendly injectable hydrogel with tunable mechanical properties. Given the simplicity in producing these rather enriched hydrogels, we envision that it may be beneficial for various tissue engineering and regenerative medicine applications.

TS044
Cartilage regeneration approach based on squid chitosan scaffolds: *in-vitro* assessment

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During the past decades, marine organisms have been the focus of considerable attention as potential source of valuable materials. For instance, chitosan is a biopolymer with high potential in the biomedical field and can be produced from crustacean shells and squid pens [1]. In this sense, we propose the use of chitosan to produce scaffolds for regenerative medicine purposes. An alkaline solution was used to deproteinize squid pens and isolate β-chitin (Chaussard 2004), which was further converted into chitosan through a deacetylation reaction. Chitosan was then processed into porous structures by freeze-drying [3], where chitosan solutions (4%) were submitted to different freezing temperature of -80 °C and -196 °C. The produced structures were further submitted to neutralization methods with 4% NaHO, including in some cases a pre-washing step using ethanol/water solutions (100:0; 90:10, 80:20; 70:30 and 50:50) [4]. The morphology of scaffolds produced using either squid or commercial chitosan revealed a lamellar structure, independent of the source and/or freezing temperature. All chitosan scaffolds produced exhibited no-cytotoxic behaviour over L929 cells. To test the *in vitro* functionality of the scaffolds, cells from the mouse chondrogenic cell line ATDC-5 were seeded in the scaffolds and cultured for different time periods. Scaffolds made from squid chitosan were shown to promote better cell adhesion than commercial chitosan scaffolds and comparable or better cell proliferation. This demonstrates that squid chitosan is a valuable alternative to produce scaffolds for different applications in regenerative medicine, namely the regeneration of cartilage.
References:

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